THE FURTHER DEVELOPMENT OF *ONCHOCERCA VOLVULUS* LEUCKART IN *SIMULIUM DAMNOSUM* THEOB.

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PLATE XIX

In a previous paper Blacklock (1926) showed that the larval form of *Onchocerca volvulus* which can often be found in the skin of infected human beings, is frequently taken up by *Simulium damnosum* when feeding on such infected skin. The larvae so taken up can be found alive and active in the gut of the insect often in large numbers. It was further shown that progressive development of the larva goes on in the gut and thorax, a development which is signalized by changes of form and increase in size of the larva.

The present series of experiments was carried out in the Konno District of Sierra Leone during part of December, 1925, and January and February, 1926, using wild flies and the same infected subject as before, the 'Case 50' referred to in the account of the former experiments. Six series of flies were allowed to feed on this case; as the early stages of the larval development had already been studied very few dissections of flies were made in the first days immediately following the infecting meal. Attention was rather directed to obtaining again those more advanced stages of the development which were described as occurring in some of the flies in the previous experiments, and to ascertaining whether developmental stages beyond this could be found such as would justify the conclusion that this species of Simulium can actually transmit *O. volvulus* infection. As a result of the additional work on the subject it is now possible to add considerably to the facts recorded

in the above paper. The present experiments have not only confirmed fully the conclusions therein reached, but have also elicited evidence that the development proceeds regularly until the head of the fly is invaded by the developing worms. Not only so, but it has been possible to demonstrate that, given favourable circumstances, these fully developed cephalic forms readily escape in numbers from the anterior part of the head in the proboscis region. Although the attainment of this degree of development might in itself be accepted as conclusive evidence that the fly concerned is capable of transmitting a filarial disease, final proof of this has been sought by injecting intra- and sub-cutaneously into two *Cercopithecus* monkeys the advanced forms of larvae in the head of Simulium.

EXPERIMENT I. On December 28th, 1925, from 7.30 to 9 a.m. 42 flies were caught when feeding on Case 50 from the waist down.

EXPERIMENT II. On December 29th, from 6 to 9.30 a.m. 67 flies were caught feeding on any part of the body.

EXPERIMENT III. On December 30th, from 8 to 10 a.m. 41 flies were caught feeding on any part.

EXPERIMENT IV. On January 16th, 1926, from 8 to 10.30 a.m. 117 flies were caught feeding on any part.

EXPERIMENT V. On January 26th, from 8 to 10 a.m. and 4 to 6 p.m. 110 flies were caught feeding on any part.

EXPERIMENT VI. On February 16th, from 4 to 6 p.m. and on February 17th, from 6 to 8 a.m., and from 4 to 6 p.m. 125 flies were caught feeding on any part.

Owing to enforced absence for part of the time immediately following capture of the flies in Experiment VI, the records of death of flies up to the seventh day could not be kept; they are, therefore, omitted from the table showing the length of life of the flies after capture. The parasitic findings in those flies of this experiment which survived for this period and were subsequently dissected are, however, included in the table of dissections.

It will be seen that only in Experiment I was any restriction imposed on the flies as to which portion of the body they should bite. The subsequent experiments involved no such restriction, since it was hoped that in this way certain of the flies would obtain light infections which would permit of their surviving longer. It

was found, however, that when the patient was seated close to the ground, raised only a few inches off it, by far the majority of the flies fed on the region of the waist and downwards.

The length of life, infection rates, and ovarian development of the flies are set out in the following tables.

Length of Life.

TABLE I.

Showing the number of flies in five experiments dying on each day after the infective feed.

Number of experiment	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Lost	Killed for dissection
	Number of flies																					
I	42	6	3	2	6	2	2	1	2	4	3	6	2	2	1	...	...	...	...	...	0	0
II	67	11	4	1	5	4	4	4	11	2	1	5	11	3	0	0	0	0	0	1	0	0
III	41	8	1	3	2	3	0	1	0	3	7	10	1	0	*0	0	1	...	...	...	*1	0
IV	117	17	6	12	10	6	16	17	†9	‡5	1	...	...	...	...	...	...	...	...	...	...	†3 ‡15
V	110	24	14	9	10	5	6	8	6	9	13	6	...	...	...	...	...	...	...	...	...	...
Totals...	377	66	28	27	33	20	28	31	28	23	25	27	14	5	1	0	1	0	0	1	1	18

It is seen from Table I that no fly survived for a longer period than 19 days, although it is possible that some of those flies killed for dissection might have lived longer ; it is not without interest that the three flies which actually lived for the longest time, 19, 16, and 14 days respectively, had apparently not fed on infected skin or on blood, as infection was not present and the ova were not developed. The highest mortality among the flies on any single day was usually on the first day after capture, due possibly in part to some mechanical injury during feeding. As regards the mortality which occurs in the first week or so, if we exclude the effects of actual confinement and fungus infections which readily attacked the flies, the most important causes of mortality appeared to be hyperinfection of the fly with larvae, and the disturbance of metabolism produced by development and retention of ova. A third cause or group of causes is doubtless the influence of such factors as temperature and humidity. But in infected flies fed on blood the influence of these

last factors is so complicated by the resultant changes produced by them on ovarian and parasitic development that it is difficult to determine what influence they really have. For example, it became evident, as will be shown presently, that a low temperature affected adversely not only the development of the ovaries of the insect, but also that of larvae ingested. The effect of this retardation should therefore be to prolong the life of the infected fly; but there is some evidence that even apart from the parasitic and ovarian development, low temperature may have a deleterious effect on the fly. Some flies which had not absorbed sufficient blood to promote development of the ovaries and which had not obtained infection with larvae from the skin, died of apparently no other cause than cold. The possibility of this being the case is not excluded by the fact already referred to that the flies which lived longest after feeding happened to be uninfected and apparently had not fed on blood.

Infection of SIMULIUM DAMNOSUM *with larvae of* O. VOLVULUS.

In Table II are given the total numbers of flies used in each experiment, the total numbers dissected after the third day, the number found infected and the site of infection in the fly.

TABLE II.

Showing results of dissection of fed flies.

Number of experiment	Total flies	Dissected	Negative	Positive	Developmental forms found in	
					Thorax	Head
I	42	21	8	13	13	2
II	67	41	12	29	28	1
III	41	20	5	15	15	2
IV	117	60	18	42	33	17
V	110	29	8	21	20	6
VI	125	30	9	21	18	6
	502	201	60	141	127	34

It is seen from Table II that of 201 flies which were dissected at a period later than the third day after feeding, 127, that is 63 per cent., showed thoracic infection, while 34, that is 17 per cent., were infected in the head.

Head infection.

In the different experiments there was considerable variation not only in the rapidity of occurrence and the proportion of head infections but also in the actual number of larvae found in this region. The occurrence of infection of the head was considered established ; when on separating the head from the thorax larval forms were found protruding from or emerging from the cut posterior end of the head ; when such forms emerged from the proboscis region before or during separation of the head or were found by dissection of the tissues of the head after it had been separated ; when larvae emerged from the proboscis region spontaneously.

Lapse of time between infective feed and head infection.

The earliest day on which larvae were found in the posterior part of the head was, in the case of Experiment IV, Fly 51, five days after the infecting feed ; the earliest day on which larvae were found in the anterior portion of the head was seven days after the infecting feed. Of the 34 flies which became infected in the head, twelve had posterior infection, seventeen anterior infection, while nine had infection in the mid-region of the head. The figure for the latter region is lower than it should be because several heads which had proved infected by the escape of larvae either anteriorly or posteriorly were preserved for sectioning or used for injection and therefore were not included in the dissections in so far as the mid-head was concerned.

Temperature in relation to development of larvae in the fly.

Notable differences in the number of head infections obtained in different experiments are accounted for largely by the temperature conditions. For example, in the first three experiments, totalling 150 flies, only five infections of the head were discovered ; yet 78 of the flies survived for a period of six days or over and were dissected, and as we have seen posterior infection of the head can occur as early as five days after feeding in favourable conditions. In the fourth experiment, with a total of 117 flies, no less than seventeen infections of the head were discovered ; yet only 66 of these flies survived for a period of six days or over and were dissected.

The first three experiments were carried out in an unwalled building at the time when the cold Harmattan wind was blowing.

The absolute maximum temperature recorded was 88, the absolute minimum 49, while the average minimum during the period was 58. The fourth experiment was carried out at a time when the external temperature was much higher, and moreover the flies were not kept in the open building but in a walled building where the temperatures recorded for the period were :—absolute maximum 87, absolute minimum 63, average minimum 68. This experiment was therefore carried out at an average minimum temperature ten degrees higher than that of the first three experiments. The fifth experiment stands intermediate in average minimum temperature and also in the resultant head infections. The influence of temperature in these experiments is shown in Table III.

Table III.

Giving the total and head infections in flies 6 days or more after the infective feed.

Number of experiment	Temperature			Flies 6 days or over dissected	Total infected	Percentage	Infected in head	Percentage infected in head
	Maximum	Minimum	Average minimum					
I, II, III	88	49	58	78	53	68	5	6
IV	87	63	68	52	37	71	16	31
V	84	60	63	29	21	72	6	21

It is seen that almost equal percentages of flies in the three sets were found to be infected at the time of dissection. Numerically speaking, therefore, development had been equal in all three sets. It is when we come to consider the degree of development that we are struck by the great differences which exist, especially between set one and set two. Here the retarding influence of low temperature on the development of the parasites is very evident ; the conclusion is supported by the facts shown in the third set. It is perhaps better to speak here of the retarding effect of low temperature, than of the accelerating effect of high temperature, because in point of fact the period during which anything approaching such low temperatures have been recorded in the Konno country is limited to those one or two months of the year when the Harmattan may be blowing.

Development of ovaries and oviposition.

In Table IV are given the numbers of flies in which notes on the condition of the ovaries were made and the occurrence of infection in relation to ovarian development.

TABLE IV.

Showing ovarian development and infection of flies.

Number of experiment	Ovaries dissected	Ova developed	Flies		Ova not developed	Flies	
			Infected	Not infected		Infected	Not infected
I	17	10	7	3	7	1	6
II	36	23	22	1	13	2	11
III	19	14	14	0	5	0	5
IV	57	44	40	4	13	1	12
V	29	23	21	2	6	0	6
VI	30	24	21	3	6	0	6
	188	138	125	13	50	4	46

It will be seen that though there is a somewhat close relationship between development of the ovaries and infection it is by no means absolute. A number of flies in which the ova were fully developed presented no infection. This condition could be brought about by the fly being resistant to infection or feeding on a portion of uninfected skin, so that while it would take up blood it would fail to take up any larvae.

Oviposition in tubes.

In experiment I, one fly was found on the ninth day to have oviposited on the glass tube ; the egg mass, yellow in colour, tough and elastic in consistency, was then almost dry. There was a tendency to a linear arrangement of the eggs at the margin of the mass but the centre was several layers thick and irregular. In this case oviposition was incomplete and the fly was dead ; this was the rule where oviposition occurred during the experiments ; the fly would

be found glued by a wing or by the tip of its abdomen to a mass of eggs laid in a very haphazard manner and unless liberated at once the fly soon died ; in some cases a large number of eggs remained in the fly, while in others one or two would be found retained ; in Table V are given the figures for oviposition found in the first five experiments.

TABLE V.

Giving the dates on which oviposition occurred in tubes during the experiments.

Number of experiment	Total flies which oviposited	Earliest day after feeding and numbers laying	Latest day after feeding
I	1	9th (1)	0
II	0	...	...
III	0	...	...
IV	11	5th (3)	7th
V	2	10th (2)	...

From this table it is seen that if we group the first three experiments together as before, and compare them with number four, there appears a very pronounced difference not only with regard to the number of flies ovipositing but also in the length of time which elapses between the date of feeding and the date of oviposition. Taking the total flies it is found that of the 150 used in the first three experiments 0·6 per cent. oviposited, while of 117 used in the fourth experiment 9·0 per cent. oviposited. We have here evidence that low temperature has produced in the first three experiments a definite retardation of the ovarian development just as it does of the parasitic larval development.

Relation between ovarian and parasitic development.

From what has been said it is clear that several of the factors which influence the rapidity of development of the ovaries have also an influence on the rapidity of the development of the parasites in the insect host tissues. Especially interesting is the correspondence between the time when oviposition can occur and the time when the parasites can reach the head. For it is extremely probable, in view

of this correspondence in time of these two developmental cycles, that the fly, after having oviposited, will be in position to infect in the act of biting any individual on whom it feeds soon after oviposition.

Emergence from the head of Simulium.

On slight pressure. When the head was infected in the anterior region it was possible to see the larvae emerging into the fluid during the manipulation of the needles in separating the head from the thorax or by slight pressure on the separated head. Again, even before attempting to separate the head a slight pressure would sometimes make them escape.

Spontaneous emergence.

This interesting phenomenon was observed on three occasions, which deserve special consideration. When it was observed that head infection was present preparations were made in order to try and inoculate the head forms into monkeys. It was thought that if monkey serum was used as the fluid for dissecting purposes the larvae would have a better opportunity of infecting the animal than if other media were used. Fly No. 101 of experiment IV was killed by carbon tetrachloride and was placed beside a drop of 48-hour old monkey serum warmed to body temperature in a hollow ground slide. The slide was put under the dissecting microscope, and the fly was pushed towards the drop of serum till its head was lying in it. Before dissection of the head from the thorax could be commenced it was noticed that larvae began to emerge from the anterior part of the head into the serum of their own accord, first one, then two and three, until ten had emerged and were moving actively in the serum. The larvae came out smoothly with a gliding movement until about a tenth of the length remained in the head and then the last portion of the body was delivered more suddenly, as if expressed by muscular contraction. This observation was so remarkable that an endeavour was made to repeat it and also to check it by using saline solution instead of serum. Fly number 108 was killed and placed in the same way with its head in monkey serum warmed to body temperature ; after one minute, larvae began to emerge from the anterior part of the head. Fly 109 was killed and placed with its head in the edge of a drop of normal saline warmed to

body temperature. In five minutes' observation no larvae emerged ; the fly was then transferred to monkey serum and after the lapse of two minutes one larva emerged ; the head was separated and dissected, but no further larvae were discovered.

These experiments, few as they necessarily are, nevertheless indicate clearly that warm monkey serum exercises an attractive influence on those larvae which are present in the anterior cephalic region of the fly and are sufficiently advanced to be ready to emerge. A similar attractive influence is not exercised by warmed normal salt solution. The results suggest that the entrance of mature larvae into the skin or into a wound during the period when the insect is biting may be determined by quite other factors than mere mechanical coincidence. It is worth recalling here that all those flies from which the head forms of larvae emerged thus spontaneously into warmed monkey-serum had fed on raisin, but had not in so doing got rid of the larvae in the head, as would be expected if the injection of larvae resulted solely from the mechanical processes of biting.

Experimental inoculation.

The following are the details of the attempts to infect monkeys with the larvae in the head of Simulium.

The first monkey received on 25.1.26 in the skin and subcutaneous tissue of the right flank the heads of six *Simulium damnosum*, Numbers 102 to 107 of experiment IV, on the ninth day after the infecting feed. The previous fly 101, dissected immediately before, had the head infection referred to above, and the next fly in the series, 108, had also head infection demonstrated by spontaneous emergence of larvae into monkey serum. In making the dissections of the heads which were infected many active larvae escaped into the fluid ; this fluid containing larvae was also rubbed on to the cut edge of the skin incision in the monkey's flank.

The second monkey received on 26.2.26 into the skin of the head over the left ear the heads of eight *Simulium damnosum*, of which three were known to be infected in the head and two more in the thorax. There was, however, some little difficulty with the insertion of them and one or two were lost, so that the chances of infection appear not quite so good in this animal. Neither animal

appeared the worse for the inoculation ; at the time of writing, that is, two months after the inoculation of the first monkey, there is no evidence of infection or nodule formation.

Proboscis infection.

When larvae emerged from the proboscis they appeared at the level of the anterior margin of the labella usually to one or other side of the mid-line. They were never seen to emerge from the hypopharynx nor were they found on dissection in the salivary glands, the salivary ducts, or the common salivary duct. In flies which were fixed in alcohol at a time when larvae had begun to come out of the proboscis, and were then cleared in clove oil and mounted, larvae could be seen coiled up at the base of the labium, while others, in varying degrees of extension, could be seen reaching forward into the portion of the labium behind the labella. The labium of *Simulium damnosum* is a large structure, comprising anteriorly the thick fleshy labella and posteriorly the wide but thinner membranous portion. It is a soft, scantily chitinized organ which appears well-adapted by its structure to accommodate large larvae, and also capable, owing to the membranous nature of its walls, of providing an easy exit for them from the proboscis during the act of feeding.

Summary of developmental stages in the fly.

In Fig. 1, A—E, are illustrated the main types of larval developmental stages found in the thorax and head. It is not to be understood that these are the only forms found because there are numerous minor modifications seen in the study of a series of dissections. Nor is it to be understood that each of the forms illustrated represents the only form seen on the day when this form may predominate. It was observed for example that even as late as the seventh day forms were occasionally found which resembled the primary gut forms ; though rather larger in dimensions and paler staining than normal their state of preservation appeared so good that one could only conclude that although they had not developed yet they had survived for many days. In the same way in dissecting the thorax it was not unusual to find forms representing very different stages of development in the same thorax.

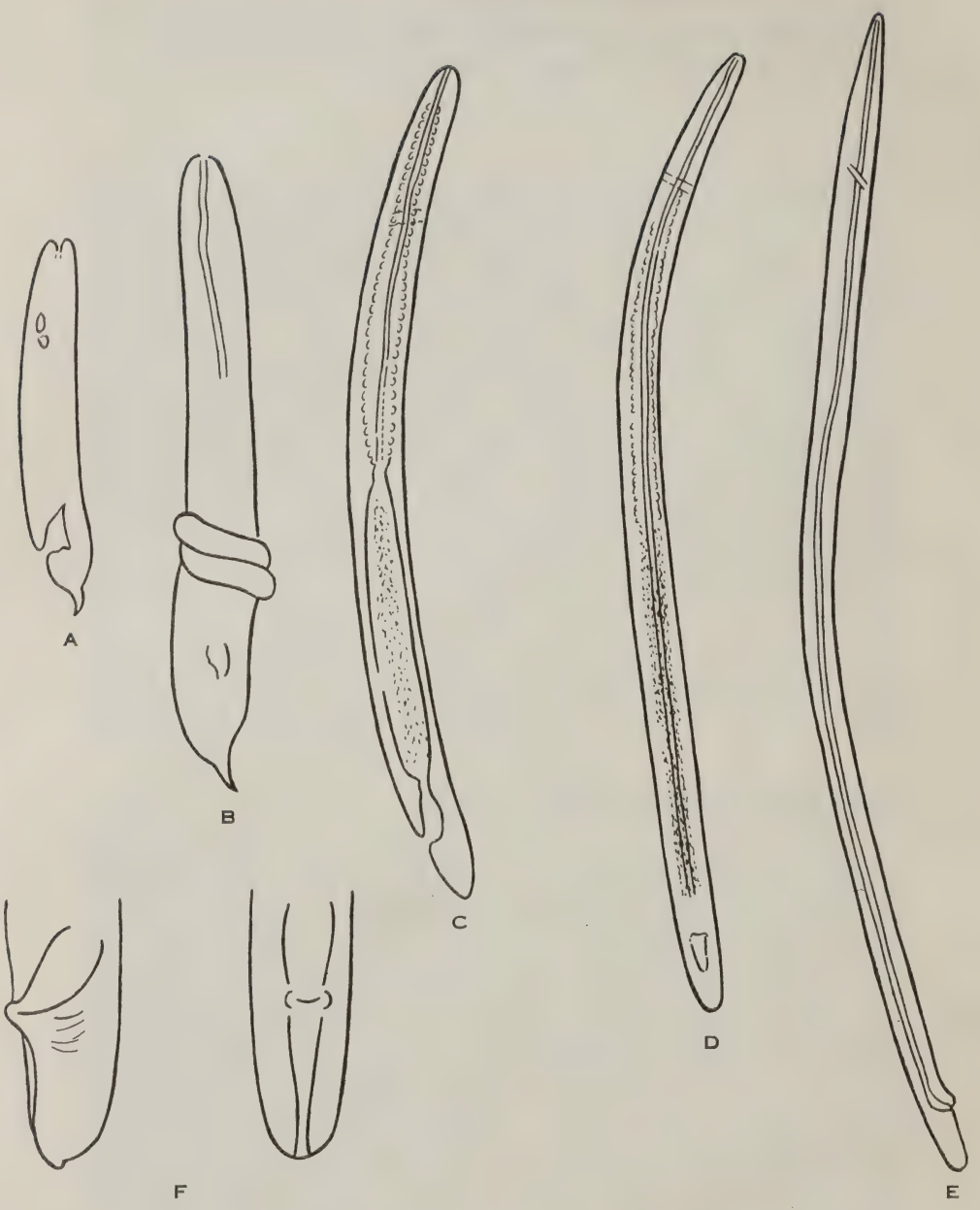

FIG. 1. *A.*—Early thoracic form, second day. *B.*—Thoracic form, undergoing ecdysis. *C.*—Advanced thoracic form, seventh day. *D.*—Slightly later thoracic form. *E.*—Proboscis form, ninth day. *F.*—Lateral and ventral views of caudal extremity of proboscis form.

The larvae taken into the gut of Simulium assume an activity which they do not possess when liberated from pieces of skin in warm water or saline solution. On the second day after ingestion forms can be found in the posterior thoracic muscles resembling in shape that seen in Fig. 1, A. They show anteriorly one or two small vacuolic areas interpreted as the excretory vesicle, and posteriorly a large vacuole opening to the exterior and often exuding a drop of clear fluid, the anus. The cells of the body in this stage are arranged more or less regularly in two sets longitudinally, a parietal and a central set, an arrangement which often gives the impression of there being a rudimentary alimentary canal, more particularly in the anterior part of the larva. These early thoracic forms are striking in shape and possess a very characteristic caudal appendage in the form of a spine which is, as a rule, straight but may be markedly curved. There is a great increase in size of these forms which seems to be accomplished, in part at least, by means of a process of ecdysis as shown in Fig. 1, B. At the conclusion of this stage there arises a more elongated larva which in place of the caudal spine has only small terminal papillae. This advanced form in the thorax occurs about the seventh day; its increase in length is accompanied by an increase in breadth; in it the alimentary canal becomes differentiated into several portions. At first it appears as in Fig 1, C, having an anterior portion of the gut with large refractile cells and a mid-portion with the walls thin and the lumen wide and filled with fine yellowish granules, followed by a short wide flask-shaped portion with the anal opening at the surface. At this stage these portions of the gut are shut off, the one from the other, by apparently impervious constricted areas at the end of the anterior and mid portions. A more advanced stage is shewn in Fig. 1, D, where the portions of the gut communicate with each other but yet in which considerable differences still exist between the portions. In Fig. 1, E, which represents a proboscis form of the ninth day, the worm is long and slender, the longest form attaining the length of over 760 μ and a width of 25 μ to 18 μ, the commonest width being 20 μ; this stage possesses a patent alimentary canal of uniform lumen which runs straight from the anterior end to a narrow slit-like anus in front of the tail. In a few individuals in addition to the fine transverse striations visible under high powers

of the microscope there were seen elevated cuticular lines separated by several striae. The nerve ring is a fairly conspicuous structure in the advanced thoracic and head forms ; in some cases a short chain of cells was seen extending backwards from the nerve ring and situated between the parietal and the gut lines of cells.

Papillae could be made out in the anal region where one was seen to be situated on each side of the anus ; these were conspicuous. Two others, of much smaller size, were distinguished at the caudal extremity and in some cases a papilla situated laterally between the anus and the caudal extremity appeared to be present. The portion of the body between the anus appears to have a groove on the ventral surface, Fig. 1, F (lateral and ventral views), the margins of the groove ending in the proximity of, or actually in, the small papillae at the caudal extremity.

SUMMARY AND CONCLUSIONS

1. Larvae of *O. volvulus* taken up from the skin by *S. damnosum* in biting undergo progressive development in the fly and finally reach the proboscis ; the time taken to complete the development depends largely upon temperature.

2. The shortest period which elapsed after feeding before the proboscis became infected was seven days.

3. The mature larvae are found in the labium of the fly and escape through the membranous portion of it.

4. In so far as experiments with wild flies can be accepted as evidence in the absence of actual transmission to man or animal, *Simulium damnosum* is a vector of *Onchocerca volvulus*.

EXPLANATION OF PLATE XIX

Mouth parts of *S. damnosum* fixed in alcohol cleared in warm clove oil and mounted; showing the position of the larvae of *O. volvulus*, emerging and *in situ*; semi-diagrammatic.

L. 1-4. Larvae of *O. volvulus*.
R. Labrum-epipharynx.
O. Labellum of Labium.
Md. Mandible.
La. Labium.
Mx. Maxilla.
P. Maxillary palp.

C. Tinling & Co., Ltd., Imp.